C1 cont'd

The present application is a National Stage of International Application No. PCT/DE98/01409, filed May 22, 1998, which claims priority to German Patent Application No. 197 21 700.1, filed May 23, 1997.

The paragraph beginning at line 5 of page 1:

OKT3 is a monoclonal IgG 2a-type antibody originating from mice, which recognizes an epitope of an ϵ-subunit of the human CD3 complex (Kung et al., Science 206, pp. 347-349 (1979); Van Wauwe, et al., J. Immunol. 124, pp. 2708-2713 (1980); Transy et al., Eur. J. Immunol. 19, pp. 947-950 (1989)). The method of obtaining the monoclonal antibody from the corresponding hybridoma is described in detail in these publications. Furthermore, the OKT3-producing hybridoma cell line was deposited by the owner of European patent 0 018 795 under ATCC No. CRL 8001 with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, on April 26, 1979. OKT3 has been used for a long time to suppress a T-cell response thus preventing the rejection of transplants (Thistlethwaite et al., Transplantation 51, pp. 1207-1212 (1991)). On the other hand, OKT3 can also trigger T-cell activation and proliferation, which stimulates the effector cells, which can be used for the adoptive cancer immunotherapy (Yannelly et al., J. Immunol. Meth. 1, pp. 91-100 (1990)). OKT3 was used as such and as a component of a bispecific antibody to direct cytotoxic T-lymphocytes against tumor cells or virus-infected cells (Nitta et al., Lancet 335, pp. 368-376 (1990); Sanna et al., Bio/Technology 13, pp. 1221-1224 (1995)). Furthermore, humanized versions of the OKT3-monoclonal antibody which were expressed in COS cells are also known (Woodle et al., J. Immunol. 148, pp. 2756-2763 (1992); Adair et al., Human. Antibod. Hybridomas, pp. 41-47 (1994)). So far there has been the problem that OKT3 has no sufficient stability and particularly cannot be expressed in known recombinant expression systems in stably fashion and sufficient amount.

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The paragraph beginning at line 10 of page 6:

Thereafter, the amplified DNA was 'blunt-end' ligated into the vector pCR-Skript SK(+) sold by the company of Stratagene, which has been cleaved using the SrfI restriction enzyme. Mutations were inserted in the V_H domain originating from OKT3 by site specific

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mutagenesis (Kunkel et al., Meth. Enzymol. 154, pp. 367-382 (1987)). The amino acid substitution at position H100A of OKT3 (exchange of cysteine for serine) was carried out using the primer SK1 5'-GTAGTCAAGGCTGTAATGATCATC (SEQ ID NO. 7).

In the Claims

1. (Amended) A recombinant antibody product, comprising the V_H domain of the OKT3 antibody, wherein the cysteine at position H100A of said V_H domain is substituted with a polar amino acid , wherein said position H100A is according to the Kabat numbering system.

2. (Amended) The recombinant antibody product , characterized in that the polar amino acid is serine.

3. (Amended) The recombinant antibody product according to claim 1 comprising the amino acid sequence depicted by SEQ ID NO:2.

4. (Amended) A method for the production of the recombinant antibody product according to any one of claims 1 to 3, characterized by the steps of:

a) obtaining mRNA from freshly subcloned hybridoma cells of OKT3 and transcription into cDNA,

b) amplifying the DNA coding for the variable domains of the light and heavy chains by means of PCR,

c) cloning of the DNA obtained in b) into a vector adapted for site-specific mutagenesis as well as introduction of a mutation in said position H100A of the V_H domain, wherein said position H100A is according to the Kabat numbering system, wherein said mutation is the substitution of a cysteine with a polar amino acid, and

d) inserting the mutated DNA obtained in c) in an expression vector and expression in a suitable expression system.